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Original Research Article

Evaluation of cardioprotective effect of *Aegle marmelos* on doxorubicin induced cardiotoxicity: an experimental study

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ABSTRACT

Background: *Aegle marmelos* (*A. marmelos*), a medicinal herb, is widely used in the Indian system of medicine for treatment of various ailments. The methanolic extract of *A. marmelos* leaves had shown antioxidant effect. However, so far aqueous extract of *A. marmelos* is not scientifically evaluated for its cardio protective potential. Hence the present study was designed to find out cardio protective role of *A. marmelos* against doxorubicin induced cardiotoxicity.
Methods: Thirty rats were randomized into five major groups (n=6). Group I received only 2ml/100g/day normal saline p.o., group II received 2ml/100g/day of normal saline p.o. followed by doxorubicin on 21st day, group III received carvedilol 30 mg/kg/day p.o., Group IV received *A. marmelos* 250mg/kg/day p.o. and Group V received *A. marmelos* 500mg/kg/day p.o. for 21days. Doxorubicin 20mg/kg i.p. single dose was given to induce cardiotoxicity in rats of group II, III, IV and V respectively on last day of experiment. Animals were sacrificed 48 hours after doxorubicin administration. Cardiac serum markers creatinine phosphokinase MB, lactate dehydrogenase, serum glutamate oxaloacetate transaminase and serum glutamate pyruvate transaminase were analysed biochemically. Histopathological changes were studied under light microscope.
Results: All cardiac serum marker levels were found significantly ($p<0.001$) increased in doxorubicin group while *A. marmelos* pre-treated group displayed significant ($p<0.001$) reduction in rise of these parameters in a dose dependent manner indicating cardio protection. Histological observations further correlated the cardio protective effect of *A. marmelos*.
Conclusions: The present study concluded that aqueous extract of *A. marmelos* possesses cardio protective potential against doxorubicin induced cardiotoxicity.

Keywords: *Aegle marmelos*, Cardio toxicity, Carvedilol, Doxorubicin

INTRODUCTION

Cardiovascular diseases (CVD's) have now become the leading cause of mortality in India. A quarter of all mortality is attributed to CVD. Ischemic heart diseases and stroke are the predominant causes and are responsible for >80% of CVD deaths.[1] The most successful treatment for acute myocardial infarction is reperfusion by early thrombolytic therapy or primary percutaneous coronary intervention (PCI), although early reperfusion can also induce injury termed as reperfusion injury. The heart can be protected against ischemia-reperfusion injury with both endogenous activation and pharmacological treatment. Pharmacological protection means that one can add a compound to the body which can protect an organ directly or by the activation of endogenous protective systems/pathways. Many agents from modern therapeutic armamentarium have been investigated for their potential to afford cardiac protection. But these agents have not been able to come up with a satisfactory answer, against the menace of cardio toxicity caused by drugs which are essentially prescribed for malignancies and other ailments. Doxorubicin induced cardiotoxicity is a well-established standard model to study the beneficial effect of many drugs

on cardiac dysfunction. Doxorubicin induced formation of reactive oxygen species (ROS) leads to oxidative stress, that results in impaired mitochondrial function, cellular membrane damage and cytotoxicity.[2]

Carvedilol, a non- selective β-blocker, had been shown to possess cardio protective effect against Doxorubicin induced cardio toxicity, that can be attributed to its antioxidant property and not to its β-blocking property.[3] In present study carvedilol served as standard drug.

Aegle marmelos, commonly known as Bael, belongs to the family Rutaceae. Medicinal properties of Aegle marmelos were well explained in Ayurveda. Aegle marmelos fruit are reported to contain several active principles like marmelosin, marmelide, luvangetin, aurapten, psoralen, and tannin. The fruit extract of Aegle marmelos have demonstrated antidiabetic, antihyperlipidemic, gastroprotective and anti- diarrhoeal, radioprotective, antimicrobial activities. Previous studies revealed preventive role of Aegle marmelos leaf extract on isoprenaline-induced MI. Though Aegle marmelos have several therapeutic properties, it has not been investigated for its cardioprotective activity against Doxorubicin induced cardiotoxicity. Hence, the present study was planned to evaluate the preventive effect of Aegle marmelos on Doxorubicin induced cardiotoxicity.

METHODS

Animals

Healthy albino Wistar rats of either sex weighing 150-200g were procured from CPCSEA approved central animal house and maintained under standard laboratory conditions (alternating periods of light and darkness of 12hr each and at 250ºC), with free access to standard rat pellet diet and tap water ad libitum. Pregnant female rats were not included in the study.

Preparation of plant extract

Leaves of *Aegle marmelos* were shade dried and coarsely powdered by using grinder mixer. The powdered material was macerated in sufficient quantity of distilled water with small quantity of chloroform to prevent fungal growth and was kept for 7 days. During maceration, it was shaken twice daily. On seventh day, it was filtered with a muselin cloth and the filtrate was concentrated on water bath (50 degree Celsius) to remove the solvent and to get sticky brown coloured extract i.e. aqueous extract of Aegle marmelos (AEAM). It was stored in sterile amber coloured storage vials in refrigerator until used for experimentation. When needed, the extract was dissolved in water and used in the study.

Drugs and chemicals

Doxorubicin hydrochloride(Zubidox) was purchased from Adley formulations Pvt Ltd, India. Carvedilol(Carca) was purchased from Intas pharmaceutical Pvt. Ltd, India. CKMB and LDH test assay kits were procured from Melrose Healthcare Pvt Ltd, India. SGOT and SGPT test assay kit were procured from ELI Tech clinical system, France.

Experimental study design

The animals were randomly divided into five groups of six animals each after obtaining approval from IAEC (Approval letter No. IAEC/2016/1 dated 28/0616). The groups are described as follows:

- *Group- I:* Control group was given 0.9 % NaCl solution in a single oral dose of 1ml/kg for 21 days.
- *Group -II:* In addition to pellet diet and and tap water ad libitum the animals of this group were treated with doxorubicin in a dose of 20 mg/kg intra-peritoneal once only on 21st day.[19]
- *Group -III:* This group was treated with Carvedilol 30 mg/kg[3] per orally for 21 days followed by administration of Doxorubicin (20 mg/kg i.p.) as in group -II.
- *Group-IV:* This group was treated with aqueous extract of *Aegle Marmelos* 250mg/kg orally for 21 days followed by administration of Doxorubicin (20 mg/kg i.p.) as in group - II.
- *Group-V:* This group was treated with aqueous extract of *Aegle Marmelos* 500mg/kg orally for 21 days followed by administration of Doxorubicin (20 mg/kg i.p.) as in group - II.

After giving Doxorubicin all animals were sacrificed under Ketamine (75mg/kg) and Diazepam (10mg/kg) anaesthesia given intra-peritoneally; forty-eight hours after doxorubicin administration.[4] Blood samples were collected from abdominal aorta for performing blood test. Also, the heart was dissected out for histopathological study.

Serum markers creatinine phosphokinase MB (CKMB), lactate dehydrogenase (LDH), serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were analysed using assay kits.

At the end of experiment the rat heart was excised and washed with normal saline. Whole of the heart was placed in 10% neutral formalin for 12-24 hours. It was then dehydrated and cleared with ethanol and xylene respectively; blocks were prepared after embedding it in paraffin wax. From these blocks sections of 5 µm thickness were made using microtome and were stained with Harris haematoxylin and Eosin stain and then subjected for histopathological examination under light microscope.[5,6]

Statistical analysis

Descriptive statistics mean, and standard error were calculated for all variables of each group to observe the

general trend of the group. The statistical analysis was carried out using one-way Analysis of variance (ANOVA) with post-hoc analysis. P-value was estimated using SPSS software. P-value <0.05 has been considered as statistically significant.

RESULTS

Serum CK-MB levels, depicted in Table 1, in normal saline treated group 1 (control group) was 0.86±0.03. It was found to be significantly increased ($p<0.001$) with administration of Doxorubicin to 12.75±0.04. Pre-treatment with Carvedilol, significantly ($p<0.001$) limited the rise in CK-MB levels after Doxorubicin administration to 1.55±0.034. With *Aegle marmelos* there is dose dependent limitation of CK-MB rise after Doxorubicin administration. Although the dose of 250 mg/kg for 21 days showed a significant limitation ($p<0.01$) of CK-MB rise (9.73±0.026) when compared to Doxorubicin treated group but it did not match the efficacy of Carvedilol treated group. However, in dose of 500 mg/kg for 21 days the *Aegle marmelos* extract had much efficacy, in limiting the CK-MB rise after Doxorubicin administration to 6.95±0.031, which was significant ($p<0.01$). A highly significant ($p<0.001$) rise in serum LDH levels was seen in doxorubicin treated group II as compared to the normal saline treated group I. The effect of Aegle marmelos pre-treatment on serum LDH levels exhibit a trend similar to that seen in case of CKMB (Table 1).

The administration of DOX significantly increased ($p<0.001$) the serum SGOT as compared to normal saline treated group. Carvedilol administration significantly reduced the rise in SGOT level ($p<0.001$). Administration of Aegle marmelos 250mg/kg was found to be statistically significant compared to Carvedilol, however *A. marmelos* 500mg/kg administration showed much more efficacy in reducing rise in SGOT levels (Table 1).

Histopathological examination

The histology of the heart tissue from rats of control group I and group III (CARVE) showed normal morphological appearances (Figures 1 and 5). While in group II (DOX) disruption, loss of myofibrils and vacuolization of the cytoplasm were observed (Figure 2). The histology of heart tissue from group IV and group V (*A. marmelos*) showed less loss of myofibrils and degree of protection was evident clearly in a dose dependent manner (Figure 3 and 4).

Table 1: Effect of carvedilol and *Aegle marmelos* in their respective doses on Doxorubicin induced changes in various biochemical parameters in albino rats (n=6).

Treatment (mg/kg)	CK-MB(IU/L) (mean±SE)	LDH (IU/L) (mean±SE)	SGOT (IU/L) (mean±SE)	SGPT (IU/L) (mean±SE)
Normal Saline	0.86±0.03	777.33±12.57	118±4.32	63.83±3.17
Doxorubicin 20	12.75±0.04^	1408.67±12.6^	245±4.70^	165±2.82^
Carvedilol 30	1.55±0.034$^{\lambda}$	874.83±13.27$^{\lambda}$	152.33±3.11$^{\lambda}$	67.68±3.92$^{\lambda}$
Aegle marmelos 250	9.73±0.026*	898.83±10.79*	169.33±4.67*	96.7±4.56*
Aegle marmelos 500	6.95±0.031*	784.50±9.01*	149.43±2.10^	66.87±2.3^

$^{£}$p< 0.05 as compared with DOX treated group.
*p< 0.01 as compared with DOX treated group.
^p< 0.001 as compared with normal saline treated group.
$^{\lambda}$p< 0.001 as compared with DOX treated group.

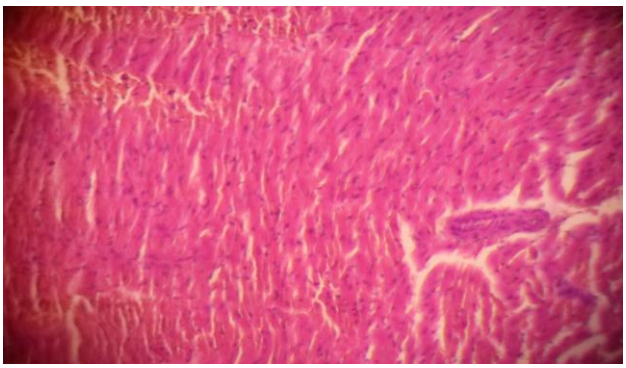

Figure 1: Microscopic features of rat heart treated with normal saline.

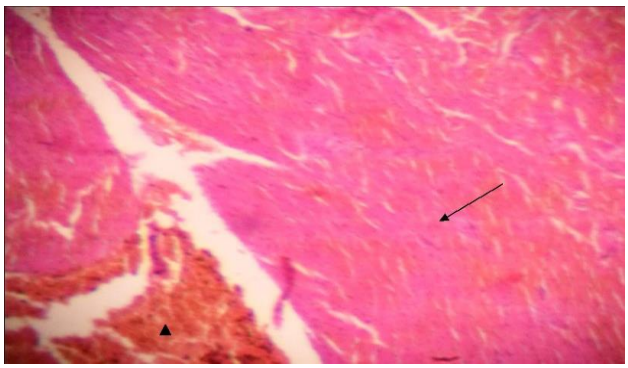

Figure 2: Microscopic features of rat heart treated with doxorubicin.

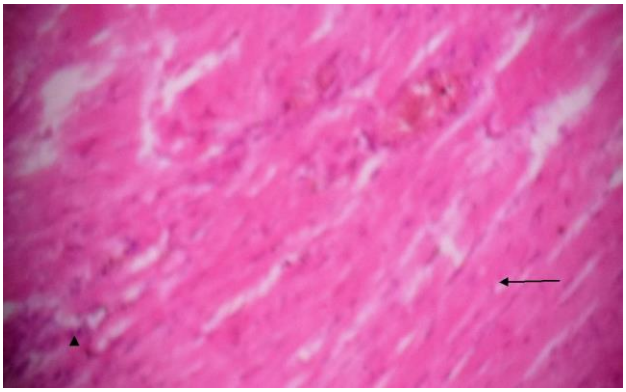

Figure 3: Microscopic features of rat heart treated with Aegle marmelos (250mg/kg/day p.o +DOX 20mg/kg i.p single dose) for 21 days.

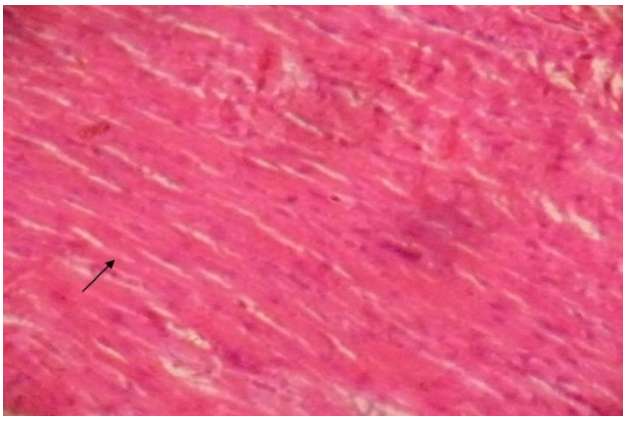

Figure 4: Microscopic features of rat heart treated with Aegle marmelos (500mg/kg/day p.o +DOX 20mg/kg i.p. single dose) for 21 days.

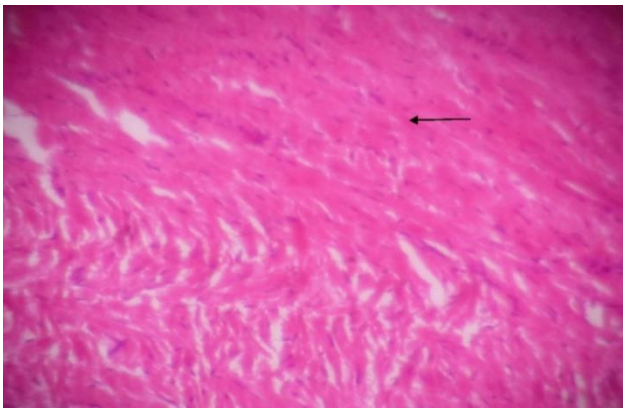

Figure 5: Microscopic features of rat heart treated with carvedilol (30mg/kg/day p.o+DOX 20 mg/kg i.p single dose) for 2 days.

DISCUSSION

Throughout study it was apparent that doxorubicin administration produced cardio toxicity in rats as examined by serum formation of free radicals and high level of oxidative stress produced by the DOX has been suggested to play an important role in promoting oxidative myocardial damage.[7] Oxidative stress has been proposed to be a cause of development and progression of myocardial infarction and heart failure.[8] The rat model of doxorubicin induced cardio toxicity has been widely used as a standard method to evaluate cardio protective drugs and to study myocardial consequences of ischemic disorders.[9,10] DOX in a single dose of 20 mg/kg intra peritoneal has been utilized to induce cardio toxicity in other studies also.[11]

Carvedilol, a non-selective beta blocker has been used as a reliable cardioprotective agent by previous researchers and in the present study carvedilol served as a standard cardio protective drug.[12]

A. marmelos contains flavinoids that have been found to have cardio protective potential against DOX induced cardio toxicity on wistar rats.[13] Myocardium contains an abundant amount of diagnostic marker enzymes for cardiotoxicity and once metabolically damaged, it releases its intracellular contents into the extracellular fluid, so that level of enzyme markers in serum reflects the alteration in membrane integrity and/or permeability.[14] Cytosolic enzymes CKMB, LDH, SGOT and SGPT which also serve as the diagnostic markers in the study, leak out from the damaged tissue to the blood stream when cell membrane becomes permeable or ruptured.[15] Results of the present study indicate that administration of DOX (20 mg/kg i.p) elevated serum levels of cardiac bio markers similarly signify the myocardial damage as demonstrated by Farvin et al.[16] This rise of serum markers was found to be statistically significant in this study also. LDH rises within 24-48 hours after a heart attack and peaks in 2-3 days in serum.[17] Consistent with the above clinical observation, in the present study we observed a significant rise in LDH levels of rats treated with DOX after 48 hours. *A. marmelos* pre-treatment significantly reduced the elevated LDH enzyme levels indicating the reduction in the severity of cardiac injury. Rise in SGOT levels has been previously reported to rise even if 10% of total myocardium damage occur and show linearity in rise with the amount of myocardial infarction.[18]

A. marmelos and carvedilol pre-treatment limited the rise in diagnostic enzyme marker level after DOX administration. It demonstrates that *A. marmelos* could maintain membrane integrity thereby restricting the leakage of these enzymes as that with carvedilol pre-treatment. The results of the serum markers correlated with the histopathological observations in the myocardial tissues of animals treated with either DOX or test drugs or normal saline. The myocardial tissue in rats of Group I treated with normal saline illustrated clear integrity of the myocardial cell membrane and absence of inflammatory cells infiltrations (Figure 1). DOX injected rats showed separation of cardiac muscle fibres and infiltration of inflammatory cells (Figure 2). The reduced inflammatory cell infiltration and normal cardiac muscle fibres architecture in *A. marmelos* treated rats further confirmed

its cardio protective effect (Figure 3 and 4). In present study, DOX induced rise of serum markers was significantly decreased by the pre-treatment of *A. marmelos* in a dose dependent manner and the maximum cardio protective features were seen with 500mg/kg/days pre-treatment, which were comparable to the standard drug, Carvedilol. The study reveals cardio- protective action of aqueous extract of *A. marmelos*, which could be attributed to synergetic effect of various antioxidant phyto-chemicals, present in it.

CONCLUSION

It was concluded from the present study that *A. marmelos* possesses cardio protective activity. These findings might prove to be helpful to understand the beneficial effects of *A. marmelos* against myocardial injury. However, an extended study using large number of animals with removal of confounding factors like pre-treatment cardiac enzyme marker levels, sequential administration of DOX and subsequent analysis is required so that substantial data can be generated for facilitating further evaluation of these agents through clinical trials.

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Ethical approval: The study was approved by the Institutional Ethics Committee (IAEC/2016/1 dated 28/0616)

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